specialized RNAi pathway may contribute to its non-cell autonomous activity, as they accumulate outside their discrete regions of biogenesis. We propose that small RNAs can possibly function as mobile inductive signals to direct patterning events during development.

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Program/Abstract # 402
Early zygotic gene regulatory network for epidermis in the ascidian *C. intestinalis*

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The epidermis is the principal interface between an animal and its environment, and generally the largest organ system. Basic epidermal cell specification in ascidians is initiated by maternal determinants. However, the early zygotic homeobox transcription factor *CiDll-B* has been shown to regulate a number of epidermal target genes, suggesting that it is a pivotal element in the epidermal cell specification network. *CiDll-B* is also one of the earliest genes to be expressed throughout, but restricted to, non-neural ectoderm. A misexpression transgene, using *CiFoxA-a* regulatory DNA to drive expression in non-epidermal territories downregulates the expression of a reporter transgene driven by the same regulatory DNA. This suggests that an additional function of *CiDll-B* may be to repress transcription of genes, such as *FoxA-a*, inappropriate to epidermis. *CiDll-B* upstream and intergenic *cis*-regulatory elements have been located which drive robust expression of reporter transgenes in the endogenous *CiDll-B* territory. This *cis*-regulatory DNA is being used to manipulate expression of a dominant negative form of *CiDll-B* to test the effect of downregulation of Dll-B on putative target genes, the aim being to build an understanding of the epidermal gene regulatory network in this simple chordate.

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Program/Abstract # 403
The *C. elegans tailless* ortholog *nhr-67* functions in uterus and tail development

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The *tailless* family of nuclear receptors is highly conserved among animals. In *Drosophila*, *tailless* (*tll*) functions in terminal embryonic patterning and central nervous system development. In vertebrates, Tlx functions in the maintenance of neural stem cell identity, but does not play a known role in terminal patterning. The *C. elegans tll* ortholog, *nhr-67*, is expressed in a dynamic pattern in pre-uterine cells. *nhr-67* is initially expressed in the 4 pre-VU cells, whose progeny form much of the uterus. *nhr-67* is upregulated in one of these four cells, the anchor cell (AC), in response to a *lin-12/Notch* reciprocal signaling system. During the L3 stage, *nhr-67* expression is maintained at high levels in the AC and at low levels in the six π cells whose twelve progeny form cells of the adult ventral uterus. Development of the π cells also depends on a *lin-12/Notch*-based signal from the AC. The development of the π cells is defective in *nhr-67* mutants, but the AC appears normal. Genetic analysis demonstrates that *nhr-67* functions downstream of *lin-12* in the ventral uterus. We are characterizing the nature of the *nhr-67* uterus defect by electron microscopy. Strong alleles of *nhr-67* arrest development in the L1 after hatching. The arrested larvae display tail defects in the hyp10 epithelial cell similar to those caused by mutations in the cadherin gene *cdh-3*. This observation suggests that a function for *tailless* in terminal development may be conserved among the ecdysozoa.

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Program/Abstract # 404
***Drosophila* CtBP causes local inhibition of Dorsal and dCBP that regulate neuroectoderm genes**

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Transcriptional repression mediated by *Drosophila* C-terminal Binding Protein (dCtBP) together with the DNA-binding repressor Snail specifies mesoderm by excluding the neuroectodermal fate in the early embryo. dCtBP interacts with Snail through the PxDLS amino acid motifs and acts as a corepressor. The Snail/dCtBP repressor complex is only able to repress adjacent activators located within 100 bp. In the last meeting, we presented the first analysis of the molecular mechanisms by which dCtBP mediates this short-range repression at the chromatin level. Particularly, we showed using chromatin immunoprecipitation (ChIP) assays that (a) the Snail/dCtBP complex locally inhibits the DNA-binding of the Dorsal activator to a neuroectodermal enhancer that are regulated by both Snail and Dorsal, but (b) recruitment of Dorsal's coactivator dCBP is not prevented, and that (c) histone H4 is, however, hypo-acetylated. These data suggested that dCtBP acts by locally preventing DNA-binding of adjacent activators, possibly by inhibiting dCBP's HAT activity. We are currently testing whether this is employed for regulation of other neuroectodermal enhancers. In addition, we are extending ChIP assays to core promoter factors to understand how the neuroectodermal enhancer when repressed by the Snail/dCtBP complex communicates with its core promoter. We are testing promoter-enhancer interaction using Chromosome Conformation Capture assay. These experiments should provide details on the molecular mechanisms of dCtBP-mediated short-range repression.

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Program/Abstract # 405
Dispensable function of the B′ regulatory subunit of Protein Phosphatase 2A (PP2A) in *Drosophila melanogaster*

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PP2A is a major serine/threonine phosphatase that has roles in diverse processes from gene regulation, protein syntheses to cytoskeleton organization. PP2A is proposed to dephosphorylate and activate the HOX protein Sex combs reduced (SCR). SCR is required for development of the larval first thoracic and labial segments, and the adult first thoracic segment and proboscis formation in *Drosophila melanogaster*. PP2A activity is composed of a set of enzymes that are composed of common catalytic and core subunit, and distinct regulator subunits. One of the regulatory subunits, B' interacts with the homeodomain of SCR (Berry and Gehring 2000). Using FLP-mediated site specific recombination I created a complete deletion of the *PP2A-B′* coding sequence. To our surprise, flies homozygous for the deletion were viable and wild type in appearance. We did not observe a large

reduction in the number of sex combs, or a proboscis to maxillary pulp transformation expected for loss of SCR activity. This indicates the PP2A-B′ activity is dispensable to development. A possible explanation for this observation is that the other PP2A activities assembled with distinct regulatory subunits compensate for the lack of PP2A-B′ activity.

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Program/Abstract # 406
Molecular fluctuations and interpreting spatial gradients, applied to Hunchback pattern formation

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Early *Drosophila* segmentation is specified by spatial gradients of transcriptional regulators, typically at hundreds to thousands of molecule copies per nucleus. Zygotic expression of segmentation genes can be affected by the fluctuations of these upstream gradients. Another, potentially greater, source of noise is the inherently stochastic nature of reactions involved in binding regulators, and transcript and protein production. From our experimentally-tested deterministic model of hunchback (*hb*) transcription under the control of Bicoid and Hb proteins, we made a stochastic version with which to investigate these issues. Real initial zygotic activation can be noisy; our simulations indicate that maternal Hb may reduce this, and could be one of its key biological functions. Multiple binding sites play a role in precise expression levels: simulations with high (wild-type) numbers of binding sites display less noise than those with lower (e.g. artificial promoter) numbers. Other flies display more or less bcd-binding sites than *D. melanogaster* (from 10 in *Musca domestica* to 4 in *D. virilis*). Our model robustly forms expression pattern across this evolutionary variation. We predict RNA pattern should be much noisier than protein pattern, and also show that Hb self-regulation can play a strong role in noise reduction. Overall, we find that the low copy numbers of both the DNA (numbers of promoter binding sites) and RNA can introduce strong noise into protein production, perhaps a more dominant effect than upstream ligand fluctuations.

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Program/Abstract # 407
Nerfin-1: A novel binding partner of Scalloped

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Scalloped (SD), a TEA/ATTS domain containing protein, is required for the proper development of *Drosophila melanogaster*. Despite being expressed in a variety of tissues, most of the work on SD has been restricted to understanding its role and function in patterning the adult wing. In the wing SD interacts with a cofactor, Vestigial (VG). Previous experiments have demonstrated that SD cannot activate transcription on its own and requires VG to form a functional transcriptional complex. The mammalian homolog of SD, TEF-1, is known to bind to several different co-factors. Work on these different cofactors has led to the identification of two SD protein:protein interaction domains: a vestigial interaction domain (VID) and a C-terminal transactivating domain. Using a series of in vivo and in vitro experiments, we demonstrate that SD interacts with the Nervous finger-1 (Nerfin-1) protein. Nerfin-1 is a Zn transcription factor that is expressed in neural precursor cells and in the eye imaginal disc. Furthermore, we show that both Nerfin-1 and VG contain a similar domain that is able to recognize and bind to the VID.

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Program/Abstract # 409
Clearing up the fog in frog embryonic blood development

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The transcription factor *Gata-1* and its cofactor *Friend of Gata* (*Fog*) are required for red blood cell (RBC) development in mice. Conversely, *Fog* acts exclusively as a negative regulator of blood in *Drosophila*. We propose to clarify its role in the evolutionary intermediate, *Xenopus laevis*. Our preliminary data suggest that in the context of RBC development, frogs are more like humans and mice than flies. Using morpholinos we have shown that knockdown of *Fog* in *Xenopus* embryos causes a reduction in primitive RBCs. Our findings are in contrast to the current model based on overexpression studies which predicts that *Xenopus Fog* inhibits RBC development by recruiting the co-repressor CtBP. To resolve these contradictory findings, we have generated a series of *Fog* mutants in which known repressor-binding domains have been disrupted. Overexpression of these mutants also results in loss of blood. Together with our morpholino studies, these data support the hypothesis that the reported loss of blood in *Fog*-overexpressing embryos is due to a dominant-negative squelching effect by which other limiting co-factors are sequestered away from the target promoter(s). To identify domains of *Fog* that are required for normal function or for squelching, we will overexpress mutant forms of *Fog* and ask whether they can rescue RBC development in *Fog* morphants, or suppress RBC development in wildtype embryos, respectively. This will allow us to identify functional domains that are required for normal erythropoiesis, or for squelching and to potentially identify novel binding partners that may be important for *Fog*'s role as an activator of blood development.

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Program/Abstract # 410
HMGA proteins in *Xenopus laevis*

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HMGA proteins are chromatin “architectural modifiers”, bearing three conserved “AT-hook” motifs with which they bind to DNA AT-rich regions to assist in gene transcription. We report the developmental expression of *Xenopus laevis* hmga2β (Xhmga2β) and ofhmgax (Xhmgax), a gene encoding a highly divergent HMGA with eight AT-hooks. Xhmga2β transcripts are first detected before the midblastula transition (MBT) by RT-PCR and then become more abundant. By in situ hybridisation (ISH), localized transcripts are first detected at neurula stages, in the presumptive central nervous system (CNS) and eye field. At tailbud and tadpole stages, Xlhmga2β mRNA is detected in the CNS, in the otic vesicles, in neural crest cell derivatives, in the notochord and